Wet and Dry Weather Toxicity in the San Gabriel River

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Abstract.—The lower San Gabriel River is an urban watershed located on the border of Los Angeles and Orange Counties. It has a diversity of potential pollutant sources including five water reclamation plants (WRPs) that discharge treated wastewaters and more than 100 storm drains that discharge largely untreated urban runoff to the river. The goal of this study was to assess the magnitude of toxicity to Ceriodaphnia dubia throughout the lower San Gabriel River watershed during wet and dry weather, identify the responsible toxicants, and compare the magnitude of toxicity over time to evaluate the effectiveness of previous watershed management actions. Wet weather runoff was sampled from sites located at the end of the four main reaches of the lower San Gabriel River; Walnut Creek, San Jose Creek, Coyote Creek, and San Gabriel River mainstem. None of the samples collected over two wet seasons exhibited acute or chronic toxicity. Dry weather samples were tested from 16 locations distributed throughout the lower watershed for up to 18 months. None of the dry weather samples from Walnut Creek, San Jose Creek, or the San Gabriel River mainstem exhibited acute or chronic toxicity. Acute and chronic toxicity was intermittently measured in the Coyote Creek tributary. Toxicity identification evaluations suggested nonpolar organic constituents, likely diazinon and perhaps surfactants, as possible toxicants. Toxicity observed in this study was significantly reduced compared to a similar study of the watershed 12 years previously, especially in the San Gabriel River mainstem. Much of the reduction in toxicity was associated with upgrades in WRP treatment. Little to no change in toxicity was observed in Coyote Creek upstream of the WRP discharge where little to no control of dry weather urban runoff had occurred.

Urban watersheds receive a multitude of potential pollutants that can affect aquatic life (Bay et al. 1996, Ackerman et al. 2005, Tiefenthaler and Stein 2005). The San Gabriel River, located on the border between Los Angeles and Orange Counties in southern California, is an ideal example of the ways in which aquatic life may be impacted by potential pollutants. Sources of potential pollutants include: 1) treated sanitary wastewaters from five Water Reclamation Plants (WRPs); 2) untreated urban runoff from approximately 350 km² of developed land discharged into the river via a municipal separate storm sewer system; and 3) once-through cooling waters from two power generating stations that is mixed with low volume industrial and sanitary wastes then discharged into the watershed's estuary.

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To complicate the fate and transport of anthropogenic pollutants and their resultant effects on aquatic life, the hydrology of many urban watersheds is often highly modified. For example, three major dams were constructed in the upper undeveloped reaches of the San Gabriel River watershed in order to capture, retain, and utilize wet season runoff for potable water use during the dry season. While this provides much needed water for the citizens of Los Angeles, the upper watershed is now hydrologically disconnected from the urbanized lower watershed. The result is that runoff from natural areas are unavailable for mixing and dispersion of anthropogenic discharges downstream. Even greater hydromodification exists in the urbanized lower San Gabriel River watershed. Many miles of the river in this portion of the watershed are lined with concrete in an effort to reduce flooding and property damage, but this modification also results in the maximum exposure of pollutants to aquatic life through the loss of natural stream and treatment processes. Where unlined channels exist in the lower watershed, temporary dams are inflated to enhance groundwater recharge.

In response to pollutant inputs and hydrologic modification, many urban watersheds have been the focus of water quality regulatory efforts. Urban Los Angeles once again provides a good example. More than 180 waterbodies in the Los Angeles region have been placed on the United States Environmental Protection Agency's (EPA's) list of impaired waters. This list, also referred to as the 303(d) list (referring to section 303 d of the Clean Water Act), identifies locations impacted by specific pollutants that can result in toxicity to aquatic life and other impacts. Virtually all of the urbanized portions of the San Gabriel River are one the 303(d) list for pollutants such as nutrients (and related impacts), certain trace metals, and aquatic toxicity. The effect of the 303(d) list is the mandate for future regulation (termed a total maximum daily load or TMDL), which will require the mitigation of these pollutant inputs.

In the San Gabriel River watershed, managers have been implementing mitigation to negate the effects of these pollutant inputs. Over the past 10 years, WRPs in the San Gabriel River watershed have installed additional treatment processes, costing over \$40 million, that have dramatically improved the water quality of their discharges for nutrients and trace metals. Controlling pollutant impacts due to urban runoff has been more difficult. Up to \$10 million has been spent on structural best management practices (BMPs) in the San Gabriel River, yet few (if any) trends in concentrations of toxic constituents monitored have been observed (LACDPW 2005). Unlike WRPs, urban runoff discharges are diffuse and, as a result, perhaps more difficult to treat and/or control.

The objective of this study was to evaluate the impact of pollutants on aquatic life in the highly urbanized lower watershed of the San Gabriel River. Impact to aquatic life was assessed through the use of toxicity testing. Four specific goals were identified: 1) assess the magnitude of toxicity at selected locations throughout the San Gabriel River watershed; 2) determine whether or not this magnitude changes seasonally; 3) if toxicity exists, identify the responsible toxicants; and 4) compare the magnitude of toxicity in this study to studies conducted historically in the San Gabriel River watershed to evaluate the effectiveness of watershed management actions.

Material and Methods

Toxicity in the San Gabriel River watershed was evaluated by separating the study into wet weather and dry weather components (Figure 1; Table 1). The wet weather component consisted of four sampling sites located at the downstream end of major



Fig. 1. Map of the lower San Gabriel River Watershed including dry and wet weather sampling locations.

reaches that receive urban runoff. Twenty-liter flow weighted composites samples were collected during three storm events on December 29, 2004 (5.3 cm precipitation), April 22, 2005 (2.2 cm precipitation), and January 1, 2006 (3.7 cm precipitation). The dry weather component consisted of sampling a total of 10 sites that included the same four sites sampled during wet weather, plus an additional six sites strategically located in the

Site #	Water Body	Location	Latitude Longitude
1	Walnut Creek	Walnut Creek At Merced Ave	N34°03′53.1″
			W117°57′09.6″
2	Walnut Creek	At Baldwin Park Blvd	N34°03'47.7"
			W117°58′54.5″
3	San Jose Creek	San Jose Creek at access gate from SJCWRP/JAO –	N34°02'06.7"
	Reach 1	Upstream of SJCWRP, approximately 100 yards downstream of Workman Mill Rd	W118°01′14.9″
4	San Gabriel River	San Gabriel River at Peck Rd - Downstream of	N34 02'02.9"
	Reach 3	confluence of SGR with SJC	W118°02'20.2"
5	San Gabriel River	San Gabriel River at Spring St - Downstream of	N33°48′38.9″
	Reach 1	LCWRP outfall 001	W118°05'26.8"
6	Coyote Creek	Coyote Creek at pedestrian foot bridge south of	N33°47′41.9″
		LBWRP - Downstream of LBWRP outfall 001 and upstream of estuary	W118°05′22.0″
7	Coyote Creek	Coyote Creek at Cerritos Ave - Upstream of LBWRP	N33°48′36.9″
		outfall; downstream of entrance of Carbon Creek into Coyote Creek	W118°04'33.3"
8	Coyote Creek	Coyote Creek at Centralia Ave - Downstream of	N33°50'19.3"
		confluence with Fullerton Creek and an industrial drain	W118°03′37.9″
9	Coyote Creek	Coyote Creek at Artesia Blvd-Downstream of Brea	N33°52′23.7″
		Creek/Coyote Creek confluence	W118°01'08.0"
10	Coyote Creek	North fork of Coyote Creek at Alondra Blvd-	N33°53′15.4″
	(North Fork)	Downstream of La Mirada Creek	W118°01′58.9″
11	Coyote Creek	Coyote Creek North Fork at La Mirada Creek/Coyote	N 33°53.731′
	(North Fork)	Creek confluence	W 118°02.155'
12	Coyote Creek	Coyote Creek North Fork- 1.0 mile upstream of	N 33°54.133′
	(North Fork)	Alondra	W 118°02.488′
13	Coyote Creek	Coyote Creek North Fork - 2.0 miles upstream of	N 33°54.862′
	(North Fork)	Alondra	W 118°02.346′
14	Coyote Creek	Coyote Creek North Fork - 2.5 miles upstream of	N 33°55.411′
	(North Fork)	Alondra	W 118°02.138'
15	La Mirada	La Mirada Creek before entering Coyote Creek North	N 33°53.503′
	Creek	Fork	W 118°01.846′
16	Milan Creek	Milan Creek before entering Coyote Creek North Fork	N 33°54.369′
			W 118°02.422′

Table 1. Station location information (NAD83 datum).

immediate vicinity of WRP discharges or urban runoff inputs. Dry weather samples were collected at least three days after rain events. Twenty-liter samples were collected from each site during dry weather on a monthly basis from March 2005 to February 2006. Within seven months of this study's initiation, an additional six sites were added for dry weather sampling, all in a single tributary (North Coyote Creek), as a result of observed toxicity. All sites from the Coyote Creek subwatershed, including the additional sites in North Coyote Creek, were sampled until August 2006.

All samples were tested for toxicity using *Ceriodaphnia dubia* examining both acute (lethality) and chronic (reproductive success) endpoints. Testing was initiated within 36 hours of sample collection using undiluted sample and a negative control following standard US EPA protocols (US EPA 1993a; Table 2). Test organisms were obtained from in-house brood cultures and test duration/exposure lasted until 60% of the surviving females in the control had released three broods (typically between six and seven days). Test solutions were renewed daily.

Test Organism:	Ceriodaphnia dubia
Organism Source:	In-house Cultures
Organism Age at Initiation:	<24 hours old and released within an eight hour period
Test Duration:	Until 60% or ore of the surviving females have three broods
Concentrations Tested:	0% and 100%
Solution Renewal:	Daily
Feeding:	0.1 ml YCT and 0.1 Selenastrum algal suspension daily
Test Chamber:	50 ml Disposable
Solution Volume:	15 ml
Control Water:	Either diluted mineral water (8 parts deionized water: 2 parts Perrier [®] water) or Reconstituted deionized water (hard)
Number of Replicates:	10
Organisms per Replicate:	1 assigned by blocking by known parentage
Photoperiod:	16 hours light (50-100 ft-c), 8 hours dark
Test Temperature:	$25 \pm 1^{\circ}$ C.
Endpoints Measured:	Survival and Reproduction
Test Acceptability Criteria:	80% or greater survival with an average of 15 or more young per surviving female in the control organisms. 60% of surviving females in the controls must produce three broods within 8 days.

Table 2. Test conditions and requirements.

Toxicity was defined as a 25%, or greater, organism response in the sample exposure relative to control organism response (i.e., <75% survival or reproduction in the 100% sample exposure). In addition, hypothesis testing was conducted following EPA guidelines (US EPA 1993a). Hypothesis testing consisted of the nonparametric Fisher's Exact Test for the survival endpoint and an analysis of variance (ANOVA) followed by a multiple comparison procedure for the reproduction endpoint. The parametric Dunnet's Test was used to identify statistically significant differences from the control for reproduction data that were normally distributed with homogeneous variances. The nonparametric Steel's Many-One Test was employed when the data failed normal distribution or equality of variance assumptions.

If a sample was toxic, a toxicity identification evaluation (TIE) was initiated (US EPA 1991, 1993b). TIE testing used the remaining sample, stored at 4°C, within seven days of baseline test conclusion. For those samples in which only the reproductive endpoint elicited a toxic response, only 100% and control concentrations were evaluated in the TIE. In these cases, the TIE consisted of a full seven-day chronic test with each sample manipulation consisting of 10 replicates, with daily renewals. For those samples where the survival endpoint elicited a toxic response, three dilutions (25%, 50%, 100%) and a control were evaluated using four replicates containing five test organisms each. In the case of a TIE in response to survival, the exposure duration was 96 hours, with renewal after 48 hours.

The TIE manipulations focused on both characterization and identification phases (EPA 1991, 1993b). These manipulations included: 1) pH adjustment; 2) aeration; 3) Ethylenedinitrilo-Tetraacetic Acid (EDTA); 4) Sodium thiosulfate (STS); 5) filtration; 6) piperonyl butoxide (PBO); 7) anion exchange column; 8) solid phase extraction (SPE); 9) SPE elution; and 10) no manipulation. By conducting each of these manipulations, the results, alone or in combination, can help to identify the responsible toxicant(s) (Table 3).

All quality assurance/quality control criteria were met for this study. These criteria included all of the test acceptability criteria (Table 2). In addition, positive control

TIE Sample Manipulation	Expected response
pH Adjustment (pH 7 and 8.5)	Alters toxicity in pH sensitive compounds (i.e., ammonia and some trace metals)
Aeration	Reduces toxicity attributable to volatile, sublatable, and/or easily oxidizable compounds
Ethylenedinitrilo-Tetraacetic Acid (EDTA) Addition	Chelates trace metals, particularly divalent, cationic metals
Sodium thiosulfate (STS) Addition	Reduces toxicants attributable to oxidants (i.e., chlorine) and some trace metals
Filtration	Removes toxicity related to and/or associated with particulates
Solid Phase Extraction (SPE) with C ₁₈	Removes toxicity associated with non-polar organics (i.e., pesticides, surfactants)
Sequential Solvent Extraction of with C_{18} Column	SPE extraction can be used to confirm toxicity due to nonpolar organic compounds. Sequential extraction using solvents of gradually decreasing polarity can separate these compounds into fractions providing further toxicant resolution and isolation for chemical analysis
Piperonyl Butoxide (PBO)	Removes toxicity caused by metabolically activated pesticides (i.e., organophosphorous pesticides). Increases toxicity attributable to pyrethroid pesticides
Anion Exchange	Removes toxicity associated with anionic compounds, including some trace metals and surfactants
No Manipulation	For comparing the relative effectiveness of other manipulations and quantifies the persistence of toxicity in the stored sample

Table 3. Toxicity Identification Evaluation (TIE) sample manipulations and their respective interpretations.

samples using reference toxicants (copper chloride) confirmed the relative sensitivity and stability of test organisms during the course of the study.

Results

None of the storms sampled during this study were acutely or chronically toxic to *Ceriodaphnia*. At all four sites, during all three storms, survival and reproduction were greater than 75% relative to controls.

Eighteen of 196 (9%) total dry weather samples exhibited chronic toxicity during this study (Table 4). Twelve of 196 (6%) total dry weather samples exhibited acute toxicity during this study. All of the dry weather samples that exhibited acute toxicity also exhibited chronic toxicity. In only one case was statistically significant toxicity observed when the response was less than 25% relative to controls (Station 15, Jan 2006). This resulted from low control variability. Only once was toxicity greater than 25% relative to controls and not statistically significant (Station 15, Mar 2006). This resulted from large sample variability.

All observed toxicity during this study was from Coyote Creek (Table 4). No toxicity was observed in Walnut Creek, San Jose Creek, or San Gabriel River Reaches 1 or 3. Widespread toxicity in Coyote Creek was observed in April 2005. As a result, an additional six stations upstream were added between July and October 2005. Widespread toxicity was observed again in August 2005. Widespread toxicity was not observed again for the remaining 12 months (September 2005 to August 2006).

In the two events for which widespread toxicity was observed in Coyote Creek (April and August 2005), the toxicity appeared to originate in the upper portions of the tributary (Figure 2). In April 2005, 100% reproductive impairment was observed at the

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Table 4.

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TOXICITY IN THE SAN GABRIEL RIVER

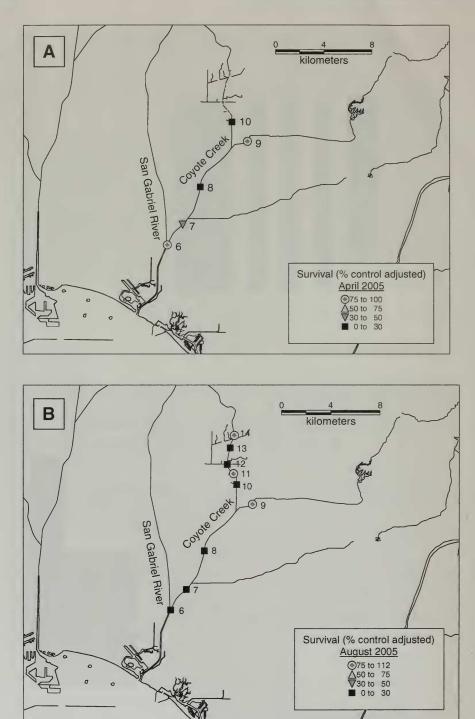


Fig. 2. Survival in Coyote Creek; A) April 2005; and B) August 2005.

TOXICITY IN THE SAN GABRIEL RIVER

					TIE R	ESULTS (Survival in	TIE RESULTS (Survival in 100% Sample)	le)			
Site #	Sample Date	No Manipulation	STS ^a	EDTA ^b	pH 7.0	pH 8.5 PBO ^c	PBO ^c	Aeration	Filtration	Centrifuge	SPE	Anion
9	Mar 2005					Sample	No longer	Toxic				
10	Mar 2005					Sample	No longer	Toxic				
10	Apr 2005	0%	0%0	0%	0%0	0%0	0%	35%	0%0	LΝ	87.5% ^e	NT
10	Jun 2005	0%0	0.000	0%0	0%0	$0_{O}^{\prime\prime}$	0% 0% 10% 10%	10%	10%	30%	100%°	100%
10	Aug 2005	0%	$0_{0}^{\prime 0}$	0%0	0%0	$0_{0}^{\prime 0}$	0.00	0%	0%0	NT	100% ^e	$0\%^{\mathfrak{l}}$
9	Sep 2005	0%0	0%0	0%0	$0_{0}^{\prime 0}$	$0_{0}^{\prime 0}$	$100\%^{g}$	0%0	0%0	LN	$100\%^{\rm e}$	0%0
15	Mar 2006					Sample	sample No longer Toxic	Toxic				
NIT - NI	-											

Table 5. Summary of dry weather TIE results.

NT = Not tested

a-Sodium thiosulfate addition, two treatments of 10 and 25 ppm

b-Ethylenedinitrilo-tetraacetic acid addition, two treatments of 25 and 50 ppm

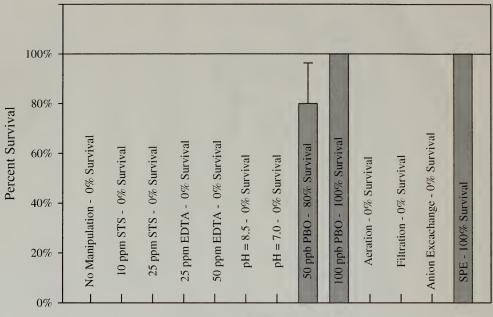
c-Piperonyl butoxide addition, two treatments of 50 and 100 ppb

d-5% survival observed in the 50 ppb treatment with 0% survival in the 100 ppb treatment

e-Toxicity recovered in only the 75% methanol elution

f-Survival observed in lower concentrations of the sample indicating partial toxicity removal

g-80% survival observed in 50 ppb treatment and 100% survival in 100 ppb treatment



Phase I Manipulations

Fig. 3. Acute Phase I TIE - site 10 sample collected on April 21, 2005.

site sampled furthest upstream (site 10) and reproductive success remained minimal moving downstream. *Ceriodaphnia* survival was also severely impacted at the furthest upstream station, then survival slowly increased downstream of the WRP discharge (Sites 7 and 6) indicating a potential dilution effect from the WRP effluent. The WRP in this reach was discharging 13 mgd of effluent to Coyote Creek upstream of Site 6 during this sampling event. In August 2005, severe reproductive impairment was again observed at the site sampled furthest upstream (site 14) and reproductive success remained minimal moving downstream. The WRP in this reach was not discharging effluent to Coyote Creek during this sampling event. *Ceriodaphnia* survival was more sporadic moving downstream during August 2005. Seventy eight percent survival was measured at site 14 and decreased to 0% survival for downstream Sites 13 and 12. Survival increased to 100% at site 11, but fell back to 0% survival for the remaining seven miles of Coyote Creek. The sudden increase in survival at Site 11 remains unexplained.

Seven TIEs were initiated during the study on dry weather samples exhibiting a 25% or greater effect (Table 5). Toxicity was no longer present for three of the samples (sites 9 and 10 March 2005, site 15 March 2006);consequently, no toxicant was identified.

Organophosphorus pesticides, most likely diazinon, were identified as the causative agent in one sample (site 10 April 2005). This result was based on the exclusive removal of toxicity using SPE and the addition of PBO, which removes non-polar organic toxicants and inhibits toxicity due to diazinon, respectively (Figure 3). The SPE was sequentially eluted and these fractions were subsequently tested. Toxicity was recovered in the 80% methanol elution of the SPE column, a fraction associated with many organophosphorus pesticides including diazinon (Figure 4). Finally, 1,700 μ g/L diazinon was quantified in the sample using Enzyme-Linked Immuno-Sorbant Assay (ELISA) techniques.

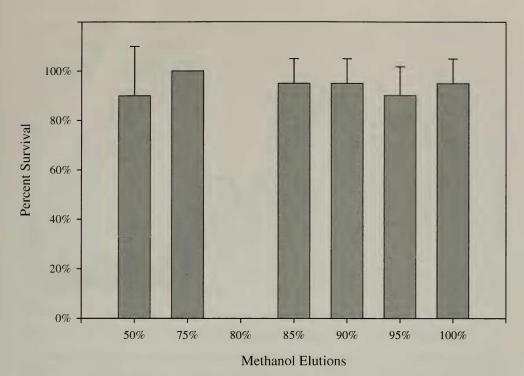
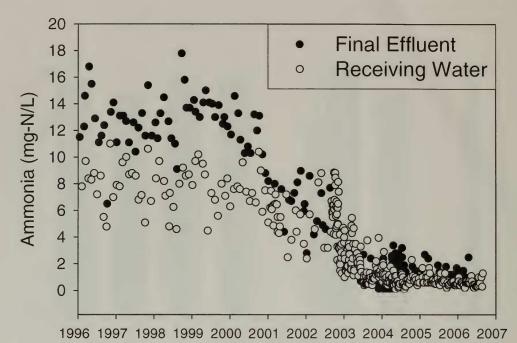


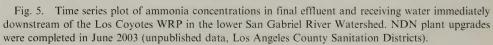
Fig. 4. Acute Phase I TIE Solid Phase Extraction Elution Testing - Site 10 sample collected on April 21, 2005.

A non-polar organic toxicant(s), possibly a surfactant(s), was identified as the causative agent in the remaining three samples (site 10 April, June, and August 2005). This result was based on the removal of toxicity using SPE. Toxicity was recovered in the 75% methanol elution, a fraction commonly associated with organophosphorus pesticides with surfactant toxicity recovery also documented (Norberg-King et al. 2005). An anion exchange column was used on two samples, with complete removal of toxicity observed in one sample (June 2005) and partial removal in the other (August 2005). This may be indicative of anionic surfactants, but might also suggest the presence of some trace metals. Elution of the anion column would help to confirm anionic surfactant toxicity, but attempts to recover toxicity from the anion column were not successful. However, other treatments to identify trace metals did not reduce toxicity (i.e., EDTA), which helps to rule-out metals as a major source of toxicity. Aeration partially removed toxicity in the April 2005 sample. Some surfactants can be removed or partially removed through aeration. Finally, PBO did not reduce toxicity, and levels of diazinon in these three samples were low (<100 μ g/L).

Discussion

Toxicity was not widespread in the San Gabriel River watershed over the 18 months examined during this study. No toxicity was observed at any site during any of the storm events sampled. Similarly, no toxicity was observed in four of the five major reaches in the lower watershed during dry weather. In Coyote Creek where toxicity was observed, the toxicity was intermittent and occurred only during six of the 18 sampling periods. This was despite an adaptive monitoring strategy, in which the number of sites sampled in Coyote Creek was doubled and the sampling period was extended by six months.





The lack of toxicity observed in this study was in direct contrast to historical studies in this watershed. While 9% of the samples were toxic in 2005/06, 55% of the samples collected for a similar study in 1992/93 were toxic (Bailey et al. 1995). Moreover, toxicity was observed in only a single reach (Coyote Creek) in 2005/06, while Bailey et al. (1995) identified toxicity in all five major reaches in the lower San Gabriel River watershed.

The difference in toxicity from tests conducted 14 years ago is likely due to changes in water quality. Bailey et al. (1995) concluded that toxicity in the San Gabriel River watershed was likely due to non-polar organics and possibly ammonia. This is not unexpected as there are multiple WRPs discharging to the San Gabriel River; these treated effluent discharges comprise roughly 80% of flow during the dry season, contributing as much as 99% of the total ammonia input (Ackerman et al., 2005). In 1992/93, ammonia levels averaged over 10 mg/L. In 2003, however, the WRPs fully implemented nitrification and denitrification treatment (NDN) processes, which subsequently reduced discharged ammonia levels more than 80% to an average of less than 2 mg/L (Figure 5). Thus, a reduction in toxicity for reaches in the San Gabriel River watershed dominated by WRP effluents can be easily explained.

The lack of toxicity observed in the current study is consistent with other toxicity data collected in recent years. In 2005, a probability-based watershed survey was conducted in the entire San Gabriel River watershed, and 7% of the stream-miles were considered toxic to *Ceriodaphnia* (Stein and Bernstein, in prep). Even this toxicity, however, was eliminated after a TIE and subsequent follow-up investigations helped identify and eliminate the illicit discharge responsible.

A second example of reduced toxicity in recent years was observed in routine toxicity monitoring required in the vicinity of the WRPs as a part of their National Pollutant

TOXICITY IN THE SAN GABRIEL RIVER

Discharge Elimination System (NPDES) permit requirements. Between June 2003 and June 2006, only 14% of the 269 total samples from 14 different sites exhibited toxicity (i.e., greater than 25% response relative to controls). For this period, toxicity was largely constrained to Coyote Creek (6% of total number of samples) and the uppermost portions of San Jose Creek (6% of total number of samples). Coyote Creek is the same tributary in which the current study found intermittent toxicity. The uppermost section of San Jose Creek was not monitored during the current study.

In contrast to the main stem of the San Gabriel River, much less effort has been spent on identifying and remediating sources of toxic pollutants in the Coyote Creek subwatershed. As a result, the toxicity in Coyote Creek has remained. The frequency of toxicity in Coyote Creek has remained similar between 1992/93 and 2004/05; roughly 12% to 22% of the samples were considered toxic. Pesticides available for application by homeowners continue to be one toxicant of concern. Diazinon was identified in 2004/05 (this study), as well as in the 1992/93 study (Bailey et al. 1995). The toxicity observed in urban runoff-dominated reaches during this study was intermittent, which is consistent with contributions by homeowner pesticide use (Schiff and Tiefenthaler 2003), illegal/ illicit discharges, and observations in other dry weather runoff toxicity studies (Greenstein et al. 2004).

Acknowledgements

Toxicity testing was conducted by the Los Angeles County Sanitation Districts (Jay P. Bottomley and the staff of the San Jose Creek Water Quality Laboratory) and Nautilus Environmental, Inc. (Chris Stransky, Howard Bailey and staff). Wet weather sampling was conducted by Mactec Engineering, Inc. (Jay Shrake and staff). Dry weather sampling was conducted by the Los Angeles County Sanitation Districts (Misty Rogalski and Jeaneal Davis) and the Southern California Coastal Water Research Project (Dario Diehl, David Tsukada, Dawn Petschauer and Diana Young). Information management was provided by Southern California Coastal Water Research Project (Jeff Brown and Larry Cooper). This project was partially supported by the Los Angeles Regional Water Quality Control Board.

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Accepted for publication 30 January 2007.